Untreated wildtype DHBV infected cells and cells co-infected with rDHBV-GFP produced equally high levels of progeny DHBV, as assessed by dot blot analysis (see Figure 5A). In contrast, approximately 20-fold less progeny DHBV (14- to 24-fold in different experiments) was released from cells coinfected with rDHBV-IFN (Figure 5A). Similar reductions (16- to 25-fold) were obtained by treatment with recombinant IFN (Figure 5A). Likewise, a strong suppression in the level of intracellular DHBV coreand L-protein was detected by Western blot analysis of cell lysates prepared at day 7 post infection (Figure 5B). Figure 5C shows a quantitative evaluation of the time course of DHBV production (DHBV-DNA equivalents). These data demonstrate that a functional cytokine expressed after hepadnaviral gene-transfer interferes with establishment of an hepadnaviral infection *in vitro*.

Example 6: Recombinant DHBV Superinfects DHBV-Infected Hepatocytes

For gene-therapeutic use in the treatment of chronic viral hepatitis, recombinant
hepadnaviruses must be able to superinfect a liver with an established viral infection.
To show that even superinfection of hepatocytes with a homologous virus is possible,
primary hepatocytes were used from productively DHBV-infected ducks which all
stained positive for DHBV S-protein, indicating productive DHBV infection.
Incubation with rDHBV-GFP at moi's ranging from 25 to 100 resulted in 1-4% of GFPpositive hepatocytes (see Figure 6). Although this transduction efficiency is
approximately 20-fold lower than the one observed with hepatocyte cultures not preinfected with DHBV, coexpression of GFP in S-protein positive cells proved that
hepatocytes with an established wildtype DHBV infection were superinfected by
rDHBV-GFP.

25

Example 7: IFN Gene Transfer Suppresses an Established DHBV Infection

To test whether hepadnaviral cytokine gene transfer was principally suited for gene-therapy for chronic hepatitis B, DHBV-positive hepatocytes were superinfected with rDHBV-IFN and monitored for the release of progeny DHBV as described above.

30 As shown in Fig. 7, DHBV production was decreased, relative to untreated controls, in a dose-dependent fashion, between 1.7 (multiplicity of infection of 25) and 4.5-fold (multiplicity of infection of 75), comparable to the effect observed by treatment with the cytokine protein at a dose showing maximal effect (4.1-fold reduction). No change in DHBV progeny production was seen upon superinfection with rDHBV-GFP, indicating that inhibition was caused by the transduced IFN gene.